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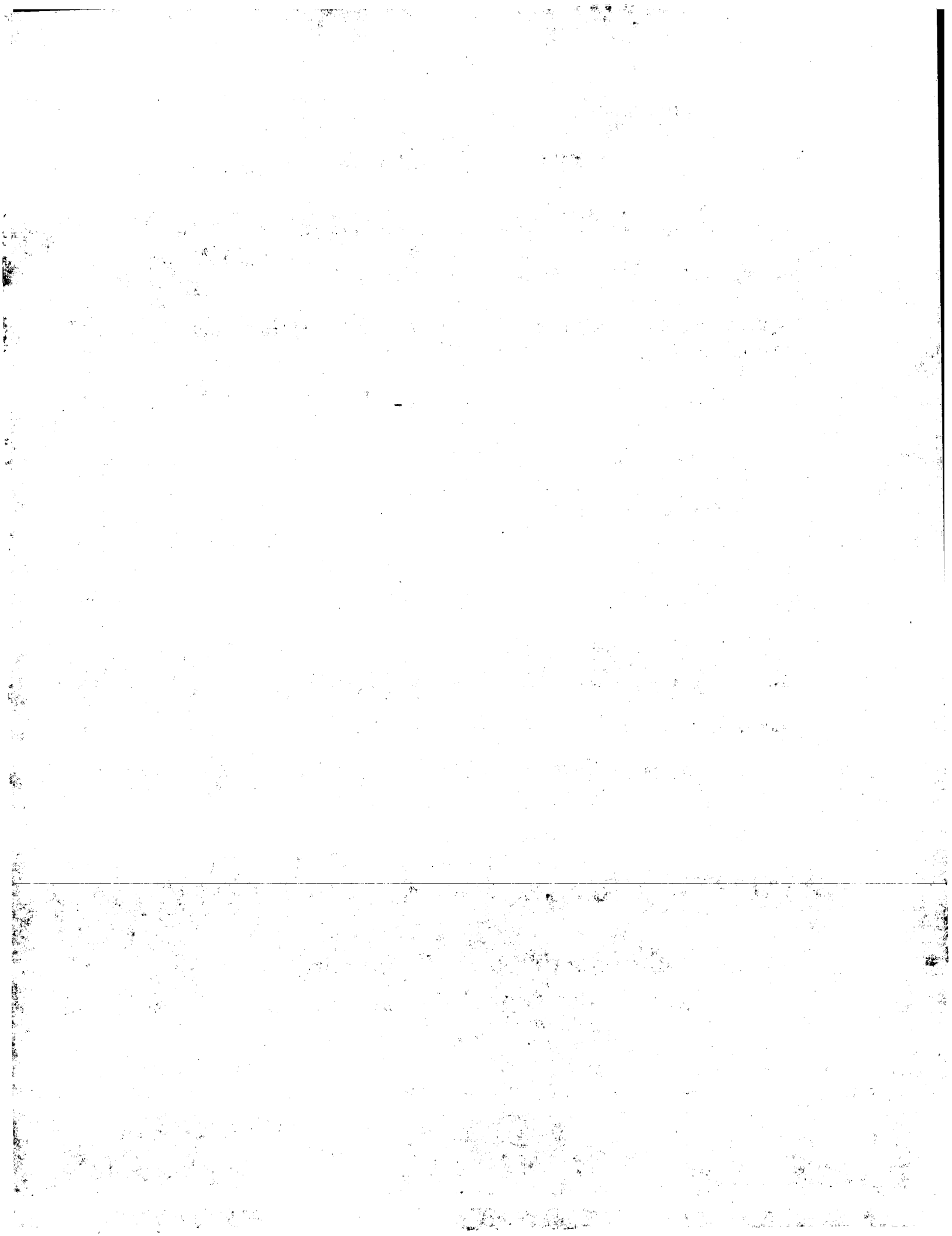
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(54) Title: NOVEL PROTEIN-KINASE, NUCLEIC ACID SEQUENCES ENCODING THE SAME AND METHODS RELATED THERETO			
(57) Abstract			
A novel protein kinase, leucine-zipper protein kinase, 668 amino acids in length is provided by the present invention. This protein kinase is localized to the human brain. Nucleic acid sequences encoding the protein kinase are also provided.			
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Anne Chavard

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NOVEL PROTEIN-KINASE, NUCLEIC ACID SEQUENCES
ENCODING THE SAME AND METHODS RELATED THERETO

Cross Reference to Related Applications

This is a continuation in part of U.S. Serial No.
5 08/205,018 filed March 1, 1994, the contents of which are
incorporated by reference herein in their entirety.

Field of the Invention

This invention is directed to a novel protein-
kinase, nucleic acid sequences encoding the same and methods
10 related thereto.

Reference to Government Grants

The work present herein was supported in part by
National Institute of Health grants NS08075, NS25044 and
NS31102. The United States government may have certain
15 rights in the invention.

Background of the Invention

Protein kinases regulate various cellular
responses to changing environmental conditions. Protein
kinases fall into two general classes: those protein kinases
20 that transfer phosphate to serine or threonine and those
proteins that transfer phosphate to tyrosine (Krebs and
Beavo, *Annu. Rev. Biochem* 48: 923-959 (1979)). A few
protein kinases, such as weel, now appear to be capable of
phosphorylating both ser/threonine and tyrosine (Lindberg et
25 al., *Trends Biochem Sci* 17: 114-119 (1992)).
Phosphorylation is of particular significance in controlling

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mitogenesis and cellular differentiation. Receptors for a number of polypeptide growth factors are transmembrane tyrosine kinases (Yarden and Ullrich, *Annu. Rev. Biochem* 57: 443-478 (1988)), which in turn stimulate serine/threonine
5 kinases such as protein kinase C, MAP kinase and p74^{ras} (Hunter et al., *Nature* 311: 480-483 (1984); Morrison et al., *Cell* 58: 649-657 (1989); Rossomondo et al., *Proc. Natl. Acad. Sci. USA* 86: 6940-6943 (1989)).

Protein kinases, and especially the overexpression
10 thereof, have been found to be linked to hyperproliferation of cells and metastasis. Many protein kinases were first identified as the products of oncogenes and still constitute the largest family of known oncogenes. Lindberg and Hunter, *Mol. and Cell. Biol.*, 10(11): 6316-6324 (1990).

15 Mutations of genes encoding members of the protein kinase family which are involved in the regulation of neuroblastic proliferation, differentiation and survival play a role in the etiology of human central nervous system tumors. Thus, it is highly desirable to gain a greater
20 understanding of this class of proteins, as well as to use such greater understanding to limit or inhibit the effects that these proteins have on cellular hyperproliferation.

Summary of the Invention

There is provided by the present invention a cDNA
25 sequence encoding a novel protein kinase, leucine-zipper protein kinase (zpk), and the protein encoded thereby.

There are provided by the present invention recombinant constructs encoding leucine-zipper protein kinase.

30 There are provided by the present invention novel methods of use and diagnosis for leucine-zipper protein kinase and cDNA coding for leucine-zipper protein kinase.

Brief Description of the Drawings

Figure 1. Nucleotide sequence (SEQ ID NO: 1) and
35 putative amino acid sequence (SEQ ID NO: 2) of leucine-

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zipper protein kinase. Amino acid numbering starts with the initiation codon.

Figure 2. Northern blots of expression of leucine zipper protein kinase in human tissue. Panel A represents Northern blots hybridized to α -[32 P] labeled leucine zipper protein kinase cDNA from human adult tissue (a) and from human fetal tissue (b). Panel B represents Northern blots hybridized to α -[32 P] labeled β -actin cDNA from human adult tissue (a) and from human fetal tissue (b).

10 Detailed Description of the Invention

A novel member of the protein serine/threonine kinase family, leucine-zipper protein kinase is provided by the present invention. As used herein, the term leucine-zipper protein kinase (zpk) refers to a protein having an amino acid sequence substantially homologous to at least a portion of the amino acid sequence set forth in SEQ ID NO: 2. In accordance with the present invention, the term "homologous" refers to a one to one correlation between the sequences of two polypeptides or oligonucleotides. Of course, 100% homology is not required in all cases. In some instances polypeptides of the present invention may be substantially homologous to the amino acid sequence set forth in SEQ ID NO: 2. Substantial homology requires only that the essential nature of the polypeptide, i.e. folding characteristics and unique features such as the leucine zipper are preserved. Thus, modifications of the leucine-zipper protein kinase are anticipated and are within the scope of the present invention. These modification may be deliberate, as through site directed mutagenesis, or may be accidental as through mutations in host which are producers of the protein. In some embodiments of the present invention polypeptides of the present invention may be at least about 75% homologous to the sequence set forth in SEQ ID NO: 2. In other embodiments of the present invention polypeptides may be at least about 85% homologous to the sequence set

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forth in SEQ ID NO: 2. In yet other embodiments of the present invention polypeptides may be at least about 95% homologous to the sequence set forth in SEQ ID NO: 2. It is also anticipated that certain non-commonly occurring amino acids may be substituted for commonly occurring counterparts to confer desirable characteristics to the resulting polypeptide.

Furthermore, it is contemplated in some aspects of the present invention that a polypeptide may comprise only a portion of the sequence set forth in SEQ ID NO: 2. This may be the case, for example, for a chimeric protein encompassing active or otherwise desirable portions of a number of proteins. A portion may also refer to a truncated polypeptide, be it substantially truncated or only slightly truncated. Such truncated polypeptides may be the result of an idiosyncrasy in the mode of production which results in truncation of amino acids from a terminal end, or a finding that the truncated polypeptide works as well or better than the full-length protein. For example, it might be found that the region directly surrounding the protein kinase domain at amino acids 231-243 is especially active.

Of course, in still other aspects of the present invention, the full-length protein, as set forth in SEQ ID NO: 2, is contemplated.

The leucine-zipper protein kinase of the present invention, depending on the pH of its environment, if suspended or in solution, or of its environment when crystallized or precipitated, if in solid form, may be in the form of pharmaceutically acceptable salts or may be in neutral form. The free amino acid groups of the protein are, of course, capable of forming acid addition salts with, for example, organic acids such as hydrochloric, phosphoric, or sulfuric acid; or with organic acids such as, for example, acetic, glycolic, succinic, or mandelic acid. The free carboxyl groups are capable of forming salts with bases, including inorganic bases such as sodium, potassium, or calcium hydroxides, and such organic bases as piperidine,

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glucosamine, trimethylamine, choline, and caffeine. In addition, the protein may be modified by combination with other biological materials such as lipids and saccharides, or by side chain modifications such as acetylation of amino groups, phosphorylation of hydroxyl side chains or oxidation of sulfhydryl groups.

The leucine-zipper protein kinase is preferably purified and isolated. "Purified" and "isolated" as the terms are used herein, are meant to refer to molecules which have been purified or synthesized so as to be substantially homogenous. The terms do not exclude the possibility that certain impurities may be present in the composition as long as the essential nature of the protein is intact.

Tissue distribution analysis indicated that leucine-zipper protein kinase is present in the brain, more so in the adult than in the fetal brain based upon the detection of a 3.4Kb mRNA transcript. A smaller mRNA transcript, about 3.2Kb was detected in kidney and skeletal muscle. Adult lung tissue expressed both transcripts at a very low level. In fetal tissue, the only definite transcript seen is in the brain. These results can be seen in Figure 2.

The cDNA sequence of a novel leucine-zipper protein kinase is also provided by the present invention. The cDNA has a long open reading frame encoding 859 amino acids. The methionine codon at nucleotides 99-101 matches Kozak's consensus sequence for the initiation of translation. Kozak *Nucleic Acid Res* 9: 5233-5252 (1981). The polyadenylation signal AATAAC was found at nucleotides 3347-3352. Wickens and Stephenson, *Science* 226: 1045-1051 (1984). The 5' cap site is CATCCG, 90 base pairs from the initiation start site.

Homology searches of leucine-zipper protein kinase with the nucleotide and amino acid databases showed no homology to any known protein kinase family. Leucine-zipper protein kinase is most similar to serine/threonine specific protein kinases. The leucine zipper protein kinase protein

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is believed to be a "non-receptor type kinase" based on its lack of a transmembrane domain. The consensus sequences for the ATP-binding site, Gly-Xaa-Gly-Xaa-Xaa-Gly and Lys residues are found at positions 537-544 and 548, 5 respectively. The protein kinase domain was found to be at position 231-243. Taylor, et al., *Annu. Rev. Cell Biol.*, 8, 429-462 (1992). Two overlapping sites of leucine zipper motif (leucine at every seventh amino acid) occur at position 442-468. A putative endoplasmic reticulum- 10 targeting sequence was located at residues 415-418 (Pelham, H.R.B., *Annu. Rev. Cell Biol.*, 5, 1-23 (1989)).

Comparison with other members of the family of protein kinases indicate that leucine-zipper protein kinase has a number of novel features. First, the glycine rich 15 loop in leucine zipper protein kinase is present towards the C-terminus of the catalytic domain, whereas in other protein kinases it is present near the N-terminus.

Endoplasmic reticulum targeting sequences (REEL) have been identified in both soluble; Pelham, H.R.B., *Annu. Rev. Cell. Biol.*, 5, 1-23 (1989); and transmembrane; 20 Jackson, et al., *EMBO J.*, 9, 3153-3162 (1990) endoplasmic reticulum proteins. A lysine rich motif at the cytoplasmically-exposed C-terminus of some transmembrane proteins was described which conferred endoplasmic reticulum 25 localization, although a more complex retention signal at the C-terminus has also be postulated. Gabathuler and Kvist, *J. Cell Biol.*, 111, 1803-1810 (1990). Leucine-zipper protein kinase contains an endoplasmic reticulum targeting sequence which is located from amino acid 415-416, rather 30 than at the extreme C-terminus of the protein.

Leucine-zipper protein kinase is also unique in that it contains a leucine-zipper motif, a sequence in which leucines occur at every seventh amino acid. Leucine-zippers contribute to targeting of various proteins (eg. glucose 35 transporters, Asano, et al., *J. Biol. Chem.*, 267, 19636-19641 (1992)) and permit dimerization of various cytoplasmic hormone receptors and enzymes. Forman, et al., *Mol*

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Endocrinol, 3, 1610-1626 (1989). Leucine zippers are also a common feature of protein transcription factors, where they permit homo- or heterodimerization resulting in tight binding to DNA strands.

5 A leucine-zipper motif has been reported only once previously in a protein kinase, the bovine cGMP-dependent protein kinase, which has a leucine-isoleucine zipper motif at its N-terminus. Wernet, et al., *FEBS*, 251, 191-196 (1989).

10 Leucine-zipper protein kinase can be routinely synthesized in substantially pure form by standard techniques well known in the art, such as commercially available peptide synthesizers and the like.

 Additionally, leucine-zipper protein kinase can be
15 efficiently prepared using any of numerous well known recombinant techniques such as those described in U.S. patent No. 4,677,063 which patent is incorporated by reference as if fully set forth herein. Briefly, most of the techniques which are used to transform cells, construct
20 vectors, extract messenger RNA, prepare cDNA libraries, and the like are widely practiced in the art, and most practitioners are familiar with the standard resource materials which describe specific conditions and procedures. However, for convenience, the following paragraphs may serve
25 as a guideline.

 Procaryotes most frequently are represented by various strains of *E. coli*. However, other microbial strains may also be used, such as *bacilli*, for example *Bacillus subtilis*, various species of *Pseudomonas*, or other
30 bacterial strains. In such procaryotic systems, plasmid vectors which contain replication sites and control sequences derived from a species compatible with the host are used. For example, *E. coli* is typically transformed using derivatives of pBR322, a plasmid derived from an *E.*
35 *coli* species by Bolivar, et al, *Gene* (1977) 2:95. pBR322 contains genes for ampicillin and tetracycline resistance, and thus provides additional markers which can be either

retained or destroyed in constructing the desired vector. Commonly used procaryotic control sequences include promoters for transcription initiation, optionally with an operator, along with ribosome binding site sequences, include such commonly used promoters as the beta-lactamase (penicillinase) and lactose (lac) promoter systems (Chang, et al., *Nature* (1977) 198:1056) and the tryptophan (trp) promoter system (Goeddel, et al. *Nucleic Acids Res* (1980) 8:4057) and the lambda derived P_L promoter and N-gene ribosome binding site (Shimatake, et al., *Nature* (1981) 292:128).

In addition to bacteria, eucaryotic microbes, such as yeast, may also be used as hosts. Laboratory strains of *Saccharomyces cerevisiae*, Baker's yeast, are most used although a number of other strains are commonly available. While vectors employing the 2 micron origin of replication are illustrated, Broach, J.R., *Meth Enz* (1983) 101:307, other plasmid vectors suitable for yeast expression are known (see, for example, Stinchcomb, et al., *Nature* (1979) 282:39, Tschempe, et al., *Gene* (1980)10:157 and Clark, L., et al., *Meth Enz* (1983) 101:300). Control sequences for yeast vectors include promoters for the synthesis of glycolytic enzymes (Hess, et al., *J Adv Enzyme Req* (1968) 7:149; Holland, et al. *Biochemistry* (1978) 17:4900). Additional promoters known in the art include the promoter for 3-phosphoglycerate kinase (Hitzeman, et al., *J Biol Chem* (1980) 255:2073), and those for other glycolytic enzymes such as glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Other promoters, which have the additional advantage of transcription controlled by growth conditions are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acidphosphatase, degradative enzymes associated with nitrogen metabolism, and enzymes responsible for maltose and galactose utilization

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(Holland, *ibid*). It is also believed terminator sequences are desirable at the 3' end of the coding sequences. Such terminators are found in the 3' untranslated region following the coding sequences in yeast-derived genes. Many of the vectors illustrated contain control sequences derived from the enolase gene containing plasmid peno46 (Holland, M.J., et al., *J Biol Chem* (1981) 256:1385) or the LEU2 gene obtained from YEpl3 (Broach, J., et al., *Gene* (1978) 8:121), however any vector containing a yeast compatible promoter, origin of replication and other control sequences is suitable.

It is also, of course, possible to express genes encoding polypeptides in eucaryotic host cell cultures derived from multicellular organisms. See, for example, *Tissue Cultures*, Academic Press, Cruz and Patterson, editors (1973). Useful host cell lines include VERO, HeLa cells, and Chinese hamster ovary (CHO) cells. Expression vectors for such cells ordinarily include promoters and control sequences compatible with mammalian cells such as, for example, the commonly used early and late promoters from Simian Virus 40 (SV 40) Fiers, et al., *Nature* (1978) 273:113), or other viral promoters such as those derived from polyoma, Adenovirus 2, bovine papilloma virus, or avian sarcoma viruses. General aspects of mammalian cell host system transformations have been described e.g. by Axel; U.S. Pat. No. 4,399,216. It now appears, also that "enhancer" regions are important in optimizing expression; these are, generally, sequences found upstream or downstream of the promoter region in non-coding DNA regions. Origins of replication may be obtained, if needed, from viral sources. However, integration into the chromosome is a common mechanism for DNA replication in eucaryotes. Plant cells are also now available as hosts, and control sequences compatible with plant cells such as the nopaline synthase promoter and polyadenylation signal sequences (Depicker, A., et al., *J Mol Appl Gen* (1982) 1:561) are available.

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Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described by Cohen, S.N., *Proc Natl Acad Sci (USA)* (1972) 5 69:2110, or methods described in *Molecular Cloning: A Laboratory Manual* (1988) Cold Spring Harbor Press, could be used for procaryotes or other cells which contain substantial cell wall barriers. Infection with *Agrobacterium tumefaciens* (Shaw, C. H., et al., *Gene* (1983) 10 23:315) is believed useful for certain plant cells. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, *Virology* (1978) 52:546 can be used. Transformations into yeast can be carried out according to the method of Van 15 Solingen, P., et al., *J Bact* (1977) 130:946 and Hsiao, C.L., et al., *Proc Natl Acad Sci (USA)* (1979) 76:3829.

cDNA or genomic libraries can be screened using the colony hybridization procedure. Generally, each microtiter plate is replicated onto duplicate nitrocellulose 20 filter papers (S&S type BA-85) and colonies are allowed to grow at 37° C. for 14-16 hr on L agar containing 50 µg/ml Amp. The colonies are lysed and DNA fixed to the filter by sequential treatment for 5 min with 500 mM NaOH, 1.5M NaCl, and are washed twice for 5 min each time with 5x standard 25 saline citrate (SSC). Filters are air dried and baked at 80° C. for 2 hr. The duplicate filters are prehybridized at 42° C. for 6-8 hr with 10 ml per filter of DNA hybridization buffer (5xSSC, pH 7.0 5x Denhardt's solution (polyvinylpyrrolidone, plus Ficoll and bovine serum albumin; 30 1x=0.02% of each), 50 mM sodium phosphate buffer at pH 7.0, 0.2% SDS, 20 µg/ml Poly U, and 50 µg/ml denatured salmon sperm DNA).

The samples can be hybridized with kinased probe under conditions which depend on the stringency desired. 35 Typical moderately stringent conditions employ a temperature of 42° C. for 24-36 hr with 1-5 ml/filter of DNA hybridization buffer containing probe. For higher

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stringencies high temperatures and shorter times are employed. Generally, the filters are washed four times for 30 min each time at 37° C. with 2xSSC, 0.2% SDS and 50 mM sodium phosphate buffer at pH 7, then are washed twice with
5 2xSSC and 0.2% SDS, air dried, and are autoradiographed at - 70° C. for 2 to 3 days.

Construction of suitable vectors containing the desired coding and control sequences employs standard ligation and restriction techniques which are well
10 understood in the art. Isolated plasmids, DNA sequences, or synthesized oligonucleotides are cleaved, tailored, and religated in the form desired.

Site specific DNA cleavage can be performed by treating the DNA with a suitable restriction enzyme (or
15 enzymes) under conditions which are generally understood in the art, and the particulars of which are specified by the manufacturer of these commercially available restriction enzymes. See, e.g., New England Biolabs, Product Catalog. In general, about 1 µg of plasmid or DNA sequence is cleaved
20 by one unit of enzyme in about 20 µl of buffer solution. Incubation times of about one hour to two hours at about 37° C. are workable, although variations can be tolerated. After each incubation, protein can be removed by extraction with phenol/chloroform, and may be followed by ether
25 extraction, and the nucleic acid recovered from aqueous fractions by precipitation with ethanol followed by running over a Sephadex G-5 spin column. If desired, size separation of the cleaved fragments may be performed by polyacrylamide gel or agarose gel electrophoresis using
30 standard techniques. A general description of size separations can be found in *Methods in Enzymology* (1980) 65:499-560.

Restriction cleaved fragments may be blunt ended by treating with the large fragment of *E. coli* DNA
35 polymerase I (Klenow) in the presence of the four deoxynucleotide triphosphates (dNTPs) using incubation times of about 15 to 25 min at 20° to 25° C. in 50 mM Tris pH 7.6,

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50 mM NaCl, 6 mM MgCl₂, 6 mM DTT and 5-10 μ M dNTPs. The Klenow fragment fills in at 5' sticky ends but chews back protruding 3' single strands, even though the four dNTPs are present. If desired, selective repair can be performed by
5 supplying only one of the, or selected, dNTPs within the limitations dictated by the nature of the sticky ends. After treatment with Klenow, the mixture is extracted with phenol/chloroform and ethanol precipitated followed by running over a Sephadex G-50 spin column. Treatment under
10 appropriate conditions with S1 nuclease results in hydrolysis of any single-stranded portion.

Synthetic oligonucleotides can be prepared by the triester method of Metteucci, et al. (*J Am Chem Soc* (1981) 103:3185) or using commercially available automated
15 oligonucleotide synthesizers. Kinasing of single strands prior to annealing or for labeling is achieved using an excess, e.g., approximately 10 units of polynucleotide kinase to 0.1 nmole substrate in the presence of 50 mM Tris, pH 7.6, 10 mM MgCl₂, 5 mM dithiothreitol, 1-2 mM ATP, 1.7
20 pmoles γ -³²P-ATP (2.9 mCi/mole), 0.1 mM spermidine, 0.1 mM EDTA.

Ligations can be performed in 15-30 μ l volumes under the following standard conditions and temperatures: 20 mM Tris-Cl pH 7.5, 10 mM MgCl₂, 10 mM DTT, 33 μ g/ml GSA,
25 10 mM-50 mM NaCl, and either 40 μ M ATP, 0.01-0.02 (Weiss) units T4 DNA ligase at 0° C. (for "sticky end" ligation) or 1 mM ATP, 0.3-0.6 (Weiss) units T4 DNA ligase at 14° C. (for "blunt end" ligation). Intermolecular "sticky end" ligations are usually performed at 33-100 μ g/ml total DNA
30 concentrations (5-100 nM total end concentration). Intermolecular blunt end ligations (usually employing a 10-30 fold molar excess of linkers) are performed at 1 μ M total ends concentration.

In vector construction employing "vector
35 fragments", the vector fragment can be treated with bacterial alkaline phosphatase (BAP) in order to remove the 5' phosphate and prevent religation of the vector. BAP

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digestions can be conducted at pH 8 in approximately 150 mM Tris, in the presence of Na⁺ and Mg²⁺ using about 1 unit of BAP per μ g of vector at 60° C. for about one hour. In order to recover the nucleic acid fragments, the preparation is
5 extracted with phenol/chloroform and ethanol precipitated and desalted by application to a Sephadex G-50 spin column. Alternatively, religation can be prevented in vectors which have been double digested by additional restriction enzyme digestion of the unwanted fragments.

10 For portions of vectors derived from cDNA or genomic DNA which require sequence modifications, site specific primer directed mutagenesis can be used. This is conducted using a primer synthetic oligonucleotide complementary to a single stranded phage DNA to be
15 mutagenized except for limited mismatching, representing the desired mutation. Briefly, the synthetic oligonucleotide is used as a primer to direct synthesis of a strand complementary to the phage, and the resulting double-stranded DNA is transformed into a phage-supporting host
20 bacterium. Cultures of the transformed bacteria are plated in top agar, permitting plaque formation from single cells which harbor the phage.

Theoretically, 50% of the new plaques will contain the phage having, as a single strand, the mutated form; 50%
25 will have the original sequence. The resulting plaques can be hybridized with kinased synthetic primer at a temperature which permits hybridization of an exact match, but at which the mismatches with the original strand are sufficient to prevent hybridization. Plaques which hybridize with the
30 probe are then picked, cultured, and the DNA recovered.

Correct ligations for plasmid construction can be confirmed by first transforming a suitable host with the ligation mixture. Successful transformants are selected by ampicillin, tetracycline or other antibiotic resistance or
35 using other markers depending on the mode of plasmid construction, as is understood in the art. Plasmids from the transformants can then be prepared according to the

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- method of Clewell, D.B., et al. *Proc Natl Acad Sci (USA)* (1969) 62:1159, optionally following chloramphenicol amplification (Clewell, D.B., *J Bacteriol* (1972) 110:667). The isolated DNA is analyzed by restriction and/or sequenced
- 5 by the dideoxy method of Snager, F., et al. *Proc Natl Acad Sci (USA)* (1977) 74:5463 as further described by Messing, et al., *F. Supp. Nucleic Acids Res* (1981) 9:309, or by the method of Maxam, et al., *Methods in Enzymology* (1980) 65:499.
- 10 In accordance with the present invention polynucleotide probes specifically hybridizable to a portion of the leucine zipper protein kinase gene are provided. Polynucleotide probes substantially homologous to a portion of the leucine-zipper protein kinase gene are also provided.
- 15 Such probes may be used for diagnostic or research purposes to detect or quantitate the expression of leucine zipper protein kinase in a sample such as by detecting the presence or absence of polynucleotide duplex formation between the polynucleotide probe and leucine-zipper protein kinase gene.
- 20 Samples may be derived from cell culture or may be derived from a patient. Samples may be biological fluids such as synovial fluid in some aspects of the invention. Tissue samples may also be used in some embodiments of the present invention. Detection of the presence of polynucleotide
- 25 duplexes is indicative of the presence of the leucine-zipper protein kinase gene in a sample and may be indicative of diseases associated with leucine zipper protein kinase, such as tumors of the central nervous system. Provision of means for detecting hybridization of polynucleotides with the
- 30 leucine-zipper protein kinase gene can routinely be accomplished. Such provision may include enzyme conjugation, radiolabelling or any other suitable detection systems. Kits for detecting the presence or absence of leucine zipper protein kinase or a particular transcript
- 35 thereof may also be prepared. Said polynucleotide probes may range in length from about 5 to about 100 nucleotide units. In more preferred embodiments of the present invention the

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probes may be from about 8 to about 75 nucleotide units in length. Ideally, said probes range in length from about 12 to about 50 nucleotide units. It is recognized that since polynucleotide probes of the present invention may

5 preferably not exceed 100 nucleotides in length, said probes may specifically hybridize to only a portion of the targeted sequence. The portion of the leucine zipper protein kinase sequence to be targeted can be identified by one skilled in the art. Most suitably, a target sequence is chosen which

10 is unique, thereby decreasing background noise attributable to hybridization by the probe other than to the target. By way of example, one skilled in the art would be unlikely to select a repeating sequence of adenine nucleotide units as this is a common sequence occurring in many genes. The

15 practitioner might choose to perform a search and comparison of sequences found in a sequence repository such as Genbank in order to identify and design a useful probe. Such methods of conventionally used to identify unique sequences. These unique sequences, when used as probes, need not

20 necessarily be crucial to the regulation of the expression of leucine-zipper protein kinase.

In accordance with other methods of the present invention, neuronal cells may be contacted with leucine-zipper protein kinase, or a portion thereof in order to

25 inhibit cellular proliferation. While not wishing to be bound to a particular theory, it is believed that the addition of exogenous leucine-zipper protein kinase, or portions thereof may interfere with specific protein-protein or protein-nucleic acid interactions involved in cellular

30 hyperproliferation. For example, by administering an inactive leucine-zipper protein kinase polypeptide or a portion thereof, it may be possible to compete with naturally occurring leucine-zipper protein kinase for binding regions of target nucleic acid molecules or

35 polypeptides in order to modulate its effect in the cell at the level of protein-protein or protein-nucleic acid interactions. In this way, it may be possible to treat a

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mammal suffering from tumors of the central nervous system by inhibiting the overexpression of leucine-zipper protein kinase *in vivo* or by interfering with a vital signal in the chain of signals leading to tumorigenicity.

5 For methods of the present invention, leucine-zipper protein kinase may be formulated into pharmacological compositions containing an effective amount of leucine-zipper protein kinase and a usual nontoxic carrier, such carriers being known to those skilled in the art. The
10 compositions may be administered by a method suited to the form of the composition. Such compositions are, for example, in the form of usual liquid preparations including solutions, suspensions, emulsions, and the like which can be given orally, intravenously, subcutaneously or
15 intramuscularly.

 The present invention is also directed to methods of inhibiting hyperproliferation of neuronal cells comprising contacting the cells with oligonucleotides substantially complementary to a portion of the nucleic acid
20 sequence set forth in SEQ ID NO: 1. "Complementary" in the context of this invention, means the ability to form hydrogen bonds, also known as Watson-Crick base pairing, between complementary bases, usually on opposite nucleic acid strands or two regions of a nucleic acid strand, to
25 form a double-stranded duplex. Guanine and cytosine are examples of complementary bases which are known to form three hydrogen bonds between them. Adenine and thymine are examples of complementary bases which are known to form two hydrogen bonds between them. "Specifically hybridizable"
30 and "substantially complementary" are terms which indicate a sufficient degree of complementarity to avoid non-specific binding of the oligonucleotide (or polynucleotide probe) to non-target sequences under conditions in which specific binding is desired, i.e., under physiological conditions in
35 the case of *in vivo* assays and therapeutic treatment, or, in the case of *in vitro* assays, under conditions in which the assays are conducted. It is understood that an

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oligonucleotide, polynucleotide probe or nucleic acid sequence need not be 100% complementary to its target nucleic acid sequence to be specifically hybridizable or effective in methods of the present invention. Thus, in
5 some embodiments of the present invention about 50% homology is envisioned. About 75% percent homology is preferred in some aspects of the present invention and about 80% homology is still more preferred. Ninety percent homology may be preferred in yet other embodiments of the present invention.
10 In the context of this invention, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid or deoxyribonucleic acid. This term includes oligomers consisting of naturally occurring bases, sugars and intersugar (backbone) linkages as well as oligomers having
15 non-naturally occurring portions which function similarly. The oligonucleotides in accordance with this invention preferably comprise from about 5 to about 50 nucleotide units. It is more preferred that such oligonucleotides comprise from about 8 to 30 nucleotide units, and still more
20 preferred to have from about 12 to 25 nucleotide units. Oligonucleotides of the present invention may be prepared by standard techniques such as solid-phase synthesis which are well known to those skilled in the art.

Furthermore, in accordance with methods of the
25 present invention, a therapeutically effective amount of oligonucleotide is administered to a mammal suffering from tumors of the central nervous system.

Oligonucleotides may be formulated in a pharmaceutical composition, which may include carriers,
30 thickeners, diluents, buffers, preservatives, surface active agents and the like in addition to the oligonucleotide. Pharmaceutical compositions may also include one or more active ingredients such as antimicrobial agents, antiinflammatory agents, anesthetics, and the like in
35 addition to oligonucleotides.

The pharmaceutical compositions of the present invention may be administered in a number of ways depending

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on whether local or systemic treatment is desired, and on the area to be treated. Administration may be done topically (including ophthalmically, vaginally, rectally, intranasally), orally, by inhalation, or parenterally, for
5 example by intravenous drip or subcutaneous, intraperitoneal or intramuscular injection.

Formulations for topical administration may include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional
10 pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms or gloves may also be useful.

Compositions for oral administration include powders or granules, suspensions or solutions in water or
15 non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be desirable.

Formulations for parenteral administration may include sterile aqueous solutions which may also contain
20 buffers, diluents and other suitable additives.

Dosing is dependent on severity and responsiveness of the condition to be treated, but will normally be one or more doses per day, with course of treatment lasting from several days to several months or until a cure is effected
25 or a diminution of disease state is achieved. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates.

The following examples are illustrative and are not meant to be limiting of the present invention.

30 Examples

Example 1

Cells

Human teratocarcinoma line NT2 was differentiated into postmitotic neurons NT2-N with retinoic acid as
35 previously described (Pleasure et al., *J. Neurosci* 12: 1802-1815 (1992); Younkin et al., *Proc. Natl. Acad. Sci. USA* 90:

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2174-2178 (1992)). Poly(A)⁺ RNA was isolated from both NT2 and NT2-N neurons using Invitrogen mRNA kit.

Example 2

Subtractive Hybridization and DNA Amplification

5 Invitrogen's Subtractor probe kit was used according to the manufacturer's instructions to isolate two different subtracted cDNAs UND and DIFF. UND was enriched in transcripts expressed in the undifferentiated stage whereas DIFF was enriched in transcripts present in the
10 neurons. One μ g portions of UND and DIFF mRNA were used for PCT amplification with degenerate primers as described in Wilks Proc. Natl. Acad. Sci. USA 86: 1603-1607 (1989). PCT was performed using a Geneamp kit (Cetus) with 1 μ g of each of the degenerate primers. The final concentration of
15 magnesium was 2.1 mM. PCT cycling was performed on a Perkin-Elmer 480 thermal cycler for 39 cycles with a profile of 1.3 minutes at 95°C (denaturation), 2 minutes at 45°C (annealing), and 2 minutes at 64°C (elongation).

Example 3

20 Subcloning of Amplified DNAs and DNA Sequencing

 The PCR reaction mixture were run on 4% Nusieve agarose gel and the amplified band of ~220 bp was excised. The band was purified using Magic PCR Kit (Promega). The amplified DNA was digested with the restriction enzymes
25 BamH1/EcoR1. The amplified DNAs were subcloned into the BamH1 and EcoR1 cleaved Bluescript DNA. A total of about 200 clones (100 representing UND and 100 representing DIFF) were examined by sequencing using a Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems). Plasmid DNA was
30 isolated using Qiagen column 20. The cycle sequencing reactions were performed in a Perkin-Elmer 480 thermal cycler for 25 cycles with a profile of 96°C for 30 seconds, 40°C for 15 seconds, and 60°C for 4 minutes. Following separation of the extension products on a Select-D G-50
35 column (5 Prime 3 Prime) the reaction mixtures were dried,

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resuspended in 4 μ l of 5:1 formamide/50 mM EDTA, loaded on a 6% sequencing gel, and analyzed using an Applied Biosystems 373 fluorescent sequencer.

Example 4**5 cDNA Library Screening**

The 210 bp 10.2 PCR clone from undifferentiated clones was radiolabelled with [³²P]dCTP and used to probe -10⁶ plaques from an amplified human fetal brain library (Stratagene) to obtain larger cDNA clones. Hybridization
10 was carried out overnight at 42°C in 50% Formamide, 5xSSPE, 5xDenhardt's, 1% SDS, 100ug/ml sheared salmon sperm DNA, and 1x10⁶ cpm/ml of probe. Filters were washed at 60°C twice in 2xSSC containing 0.1% SDS, and exposed overnight to Kodak XAR-5 film at -70°C.

15 Example 5**Sequence Determination**

cDNAs were subcloned into a plasmid vector BluescriptSk. For complete sequence determination, unidirectional nested deletions was performed using the
20 ExoIII/Mung Bean nuclease kit from Stratagene. The colonies obtained after deletions were sequenced as described earlier using a Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems). The DNA sequence obtained was determined after sequencing twice.

25 Example 6**Sequence comparisons**

All sequence manipulations were done on a VAX using the University of Wisconsin Genetics Computer Group Sequence Analysis Software Package. DNA fragments obtained
30 after nested deletions was assembled into Contigs using the Programme Sequencer 2.0 (Gene Codes Corp). Protein analysis was done using MacVector (IBI).

Example 7**RNA Analysis**

Human multiple tissue northern blots were purchased from Clontech laboratories. Hybridization conditions were similar to that used for library screening. Filters were washed to a final stringency of 0.1 x SSC/0.1% SDS at 65°C before exposure to XAR-5 x-ray film.

Example 8**Localization of the zpk Gene to Chromosome 12**

Fluorescence *in situ* hybridization (FISH) and somatic cell hybrid analysis were used to localize the *zpk* gene to chromosome 12 of the human genome. Briefly, a *zpk* clone, ZPK P1, was obtained using two oligonucleotides CCATGAAAGCCACTCGTATTCCT (SEQ ID NO:3) and TCTTTCTGTTGATTATGTGGCGC (SEQ ID NO:4), both of which correspond to the 3' untranslated region. This clone, ZPK P1, was shown to contain the *zpk* sequence by digesting the plasmid with EcoRI and HindIII and by Southern analysis with [α -³²P]-labeled ZPK cDNA.

To map the *zpk* gene, FISH and somatic cell hybrid analysis were performed. The ZPK P1 clone was labeled by nick-translation with digoxigenin-11-dUTP using a large fragment labeling kit from Oncor (Gaithersburg, MD). Human metaphase chromosome spreads were prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes from normal controls according to routine methods. Labeled ZPK (200 ng) and Cot-1 DNA (20 μ g) were dissolved in 15 μ l of Hybrisol VII (Oncor), denatured for 5 minutes at 70°C, preannealed at 37°C for 30 minutes, and applied to slides that had been denatured at 70°C for 2 minutes. The slides were hybridized overnight at 37°C and then rinsed at 39°C in 50% formamide/1X SSC for 10 minutes, followed by two washes in 2X SSC for 2 minutes each. Detection of probes was accomplished with anti-digoxigen rhodamine (Oncor). Cells were counterstained with DAPI/antifade (Oncor) and viewed with a Zeiss axiophot microscope equipped with a triple band

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pass filter set. A total of 50 metaphase cells were examined in replicate experiments and demonstrated signals on one or both chromatids for both chromosomes 12 in all metaphases examined.

5 To confirm independently the map location for *zpk* to chromosome 12, ZPK cDNA was hybridized to a somatic cell hybrid mapping panel. The panel consisted of the following cell lines and chromosomal number (in parenthesis): GM10880 (1, 3, 14); GM10826B (2); GM10253 (3); GM10115 (4); GM10114
10 (5); GM10629 (6); GM10791 (7); GM10156B (8); GM10611 (9); GM10926B (10); GM10927A9 (11); GM10868 (12); GM10898 (13); GM10479 (14); GM11418 (15); GM10567 (16); GM10498 (17); GM11010 (18); GM10612 (19); GM10478 (4,20); GM08854 (21); GM10888 (22); PHL-17 (X); and GM06317 (Y). The mouse cell
15 line Cl 1-D and chinese hamster cell line RJK-88 were used as rodent controls.

Ten micrograms of DNA from each cell line was digested with HindIII, loaded onto a 0.8% agarose gel, subjected to electrophoresis, and blotted onto Hybond-N
20 (Amersham). The ZPK cDNA probe was labeled by the random primer method with [α -³²P] and hybridized overnight at 68°C in Church's solution (1 mM EDTA, 7% SDS, 0.5 M sodium phosphate, pH 7.2). Blots were washed for one hour in 2X SSC/0.1% SDS at 68°C and exposed to radiography film for 48
25 hours. Two bands corresponding to those seen on Southern blots from normal human genomic DNA were observed in GM10868, which contains chromosome 12 as the only human chromosome, confirming that the human *zpk* gene maps to chromosome 12.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Usharani R. Reddy, David Pleasure and the Children's Hospital of Philadelphia
- (ii) TITLE OF INVENTION: Novel Protein Kinase, Nucleic Acid Sequences Encoding the Same and Methods Related Thereto
- (iii) NUMBER OF SEQUENCES: 4
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Woodcock Washburn Kurtz Mackiewicz and Norris
 - (B) STREET: One Liberty Place - 46th Floor
 - (C) CITY: Philadelphia
 - (D) STATE: PA
 - (E) COUNTRY: U.S.A.
 - (F) ZIP: 19103
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: 3.5 inch disk, 720 Kb
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: WordPerfect 5.1
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: N/A
 - (B) FILING DATE: herewith
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/205,018
 - (B) FILING DATE: 01-MAR-1994
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Rebecca L. Ralph (formerly Gaumond)
 - (B) REGISTRATION NUMBER: 35,152
 - (C) REFERENCE/DOCKET NUMBER: CH-0488
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 215-568-3100
 - (B) TELEFAX: 215-568-3439

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3389 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 99..2105
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

AGCATCCGGA GCGGAGCTGC AGCAGCGCCG CCTTTTGTGC TGC GGCCGCG GAGCCCCCGA 60
GGGCCCCAGTG TTCACCATCA TACCAGGGGC CAGAGGCG ATG GCT TGC CTC CAT      113
                                     Met Ala Cys Leu His
                                     1          5

GAG ACC CGA ACA CCC TCT CCT TCC TTT GGG GGC TTT GTG TCT ACC CTA      161
Glu Thr Arg Thr Pro Ser Pro Ser Phe Gly Phe Val Ser Thr Leu
                10                15                20

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AGT GAG GCA TCC ATG CGC AAG CTG GAC CCA GAC ACT TCT GAC TGC ACT	209
Ser Glu Ala Ser Met Arg Lys Leu Asp Pro Asp Thr Ser Asp Cys Thr	
25 30 35	
CCC GAG AAG GAC CTG ACG CCT ACC CAT GTC CTG CAG CTA CAT GAG CAG	257
Pro Glu Lys Asp Leu Thr Pro Thr His Val Leu Gln Leu His Glu Gln	
40 45 50	
GAT GCA GGG GGC CCA GGG GGA GCA GCT GGG TCA CCT GAG AGT CGG GCA	305
Asp Ala Gly Gly Pro Gly Gly Ala Ala Gly Ser Pro Glu Ser Arg Ala	
55 60 65	
TCC AGA GTT CGA GCT GAC GAG GTG CGA CTG CAG TGC CAG AGT GGC AGT	353
Ser Arg Val Arg Ala Asp Glu Val Arg Leu Gln Cys Gln Ser Gly Ser	
70 75 80 85	
GGC TTC CTT GAG GGC CTC TTT GGC TGC CTG CGC CCT GTC TGG ACC ATG	401
Gly Phe Leu Glu Gly Leu Phe Gly Cys Leu Arg Pro Val Trp Thr Met	
90 95 100	
ATT GGC AAA GCC TAC TCC ACT GAG CAC AAG CAG CAG CAG GAA GAC CTT	449
Ile Gly Lys Ala Tyr Ser Thr Glu His Lys Gln Gln Gln Glu Asp Leu	
105 110 115	
TGG GAG GTC CCC TTT GAG GAA ATC CTG GAC CTG CAG TGG GTG GGC TCA	497
Trp Glu Val Pro Phe Glu Glu Ile Leu Asp Leu Gln Trp Val Gly Ser	
120 125 130	
GGG GCC CAG GGT GCT GTC TTC CTG GGG CGC TTC CAC GGG GAG GAG GTG	545
Gly Ala Gln Gly Ala Val Phe Leu Gly Arg Phe His Gly Glu Glu Val	
135 140 145	
GCT GTG AAG AAG GTG CGA GAC CTC AAA GAA ACC GAC ATC AAG CAC TTG	593
Ala Val Lys Lys Val Arg Asp Leu Lys Glu Thr Asp Ile Lys His Leu	
150 155 160 165	
CGA AAG CTG AAG CAC CCC AAC ATC ATC ACT TTC AAG GGT GTG TGC ACC	641
Arg Lys Leu Lys His Pro Asn Ile Ile Thr Phe Lys Gly Val Cys Thr	
170 175 180	
CAG GCT CCC TGC TAC TGC ATC CTC ATG GAG TTC TGC GCC CAG GGC CAG	689
Gln Ala Pro Cys Tyr Cys Ile Leu Met Glu Phe Cys Ala Gln Gly Gln	
185 190 195	
CTG TAT GAG GTA CTG CGG GCT GGC CGC CCT GTC ACC CCC TCC TTA CTG	737
Leu Tyr Glu Val Leu Arg Ala Gly Arg Pro Val Thr Pro Ser Leu Leu	
200 205 210	
GTT GAC TGG TCC ATG GGC ATC GCT GGT GGC ATG AAC TAC CTG CAC CTG	785
Val Asp Trp Ser Met Gly Ile Ala Gly Gly Met Asn Tyr Leu His Leu	
215 220 225	
CAC AAG ATT ATC CAC AGG GAT CTC AAG TCA CCC AAC ATG CTA ATC ACC	833
His Lys Ile Ile His Arg Asp Leu Lys Ser Pro Asn Met Leu Ile Thr	
230 235 240 245	
TAC GAC GAT GTG GTG AAG ATC TCA GAT TTT GGC ACT TCC AAG GAG CTG	881
Tyr Asp Asp Val Val Lys Ile Ser Asp Phe Gly Thr Ser Lys Glu Leu	
250 255 260	
AGT GAC AAG AGC ACC AAG ATG TCC TTT GCA GGG ACA GTA GCC TGG ATG	929
Ser Asp Lys Ser Thr Lys Met Ser Phe Ala Gly Thr Val Ala Trp Met	
265 270 275	
GCC CCT GAG GTG ATC CGC AAT GAA CCT GTG TCT GAG AAG GTC GAC ATC	977
Ala Pro Glu Val Ile Arg Asn Glu Pro Val Ser Glu Lys Val Asp Ile	
280 285 290	

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TGG	TCC	TTT	GGC	GTG	GTG	CTA	TGG	GAA	CTG	CTG	ACT	GGT	GAG	ATC	CCC	1025
Trp	Ser	Phe	Gly	Val	Val	Leu	Trp	Glu	Leu	Leu	Thr	Gly	Glu	Ile	Pro	
295						300					305					
TAC	AAA	GAC	GTA	GAT	TCC	TCA	GCC	ATT	ATC	TGG	GGT	GTG	GGA	AGC	AAC	1073
Tyr	Lys	Asp	Val	Asp	Ser	Ser	Ala	Ile	Ile	Trp	Gly	Val	Gly	Ser	Asn	
310					315					320					325	
AGT	CTC	CAT	CTG	CCC	GTG	CCC	TCC	AGT	TGC	CCA	GAT	GGT	TTC	AAG	ATC	1121
Ser	Leu	His	Leu	Pro	Val	Pro	Ser	Ser	Cys	Pro	Asp	Gly	Phe	Lys	Ile	
				330					335					340		
CTG	CTT	CGC	CAG	TGC	TGG	AAT	AGC	AAA	CCA	CGA	AAT	CGC	CCA	TCA	TTC	1169
Leu	Leu	Arg	Gln	Cys	Trp	Asn	Ser	Lys	Pro	Arg	Asn	Arg	Pro	Ser	Phe	
			345					350					355			
CGA	CAG	ATC	CTG	CTG	CAT	CTG	GAC	ATT	GCC	TCA	GCT	GAT	GTA	CTC	TCC	1217
Arg	Gln	Ile	Leu	Leu	His	Leu	Asp	Ile	Ala	Ser	Ala	Asp	Val	Leu	Ser	
		360					365					370				
ACA	CCC	CAG	GAG	ACT	TAC	TTT	AAG	TCC	CAG	GCA	GAG	TGG	CGG	GAA	GAA	1265
Thr	Pro	Gln	Glu	Thr	Tyr	Phe	Lys	Ser	Gln	Ala	Glu	Trp	Arg	Glu	Glu	
	375						380				385					
GTA	AAA	CTG	CAC	TTT	GAA	AAG	ATT	AAG	TCA	GAA	GGG	ACC	TGT	CTG	CAC	1313
Val	Lys	Leu	His	Phe	Glu	Lys	Ile	Lys	Ser	Glu	Gly	Thr	Cys	Leu	His	
390					395					400					405	
CGC	CTA	GAA	GAG	GAA	CTG	GTG	ATG	AGG	AGG	AGG	GAG	GAG	CTC	AGA	CAC	1361
Arg	Leu	Glu	Glu	Glu	Leu	Val	Met	Arg	Arg	Arg	Glu	Glu	Leu	Arg	His	
				410					415					420		
GCC	CTG	GAC	ATC	AGG	GAG	CAC	TAT	GAA	AGG	AAG	CTG	GAG	AGA	GCC	AAC	1409
Ala	Leu	Asp	Ile	Arg	Glu	His	Tyr	Glu	Arg	Lys	Leu	Glu	Arg	Ala	Asn	
			425					430					435			
AAC	CTG	TAT	ATG	GAA	CTT	AAT	GCC	CTC	ATG	TTG	CAG	CTG	GAA	CTC	AAG	1457
Asn	Leu	Tyr	Met	Glu	Leu	Asn	Ala	Leu	Met	Leu	Gln	Leu	Glu	Leu	Lys	
		440					445					450				
GAG	AGG	GAG	CTG	CTC	AGG	CGA	GAG	CAA	GCT	TTA	GAG	CGG	AGG	TGC	CCA	1505
Glu	Arg	Glu	Leu	Leu	Arg	Arg	Glu	Gln	Ala	Leu	Glu	Arg	Arg	Cys	Pro	
	455					460					465					
GGC	CTG	CTG	AAG	CCA	CAC	CCT	TCC	CGG	GGC	CTC	CTG	CAT	GGA	AAC	ACA	1553
Gly	Leu	Leu	Lys	Pro	His	Pro	Ser	Arg	Gly	Leu	Leu	His	Gly	Asn	Thr	
	470				475					480					485	
ATG	GAG	AAG	CTT	ATC	AAG	AAG	AGG	AAT	GTG	CCA	CAG	AAT	CTG	TCA	CCC	1601
Met	Glu	Lys	Leu	Ile	Lys	Lys	Arg	Asn	Val	Pro	Gln	Asn	Leu	Ser	Pro	
				490					495					500		
CAT	AGC	CAA	AGG	CCA	GAT	ATC	CTC	AAG	GCG	GAG	TCT	TTG	CTC	CCT	AAA	1649
His	Ser	Gln	Arg	Pro	Asp	Ile	Leu	Lys	Ala	Glu	Ser	Leu	Leu	Pro	Lys	
			505					510					515			
CTA	GAT	GCA	GCC	CTG	AGT	GGG	GTG	GGG	CTT	CCT	GGG	TGT	CCT	AAG	GCC	1697
Leu	Asp	Ala	Ala	Leu	Ser	Gly	Val	Gly	Leu	Pro	Gly	Cys	Pro	Lys	Ala	
			520				525					530				
CCC	CCC	TCA	CCA	GGA	CGG	AGT	CGC	CGT	GGC	AAG	ACC	CGT	CAC	CGC	AAG	1745
Pro	Pro	Ser	Pro	Gly	Arg	Ser	Arg	Arg	Gly	Lys	Thr	Arg	His	Arg	Lys	
			535				540				545					
GCC	AGC	GCC	AAG	GGG	AGC	TGT	GGG	GAC	CTG	CCT	GGG	CTT	CGT	ACA	GCT	1793
Ala	Ser	Ala	Lys	Gly	Ser	Cys	Gly	Asp	Leu	Pro	Gly	Leu	Arg	Thr	Ala	
	550				555					560					565	

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GTG	CCA	CCC	CAT	GAA	CCT	GGA	GGA	CCA	GGA	AGC	CCA	GGG	GGC	CTA	GGA	1841
Val	Pro	Pro	His	Glu	Pro	Gly	Gly	Pro	Gly	Ser	Pro	Gly	Gly	Leu	Gly	
				570					575					580		
GGG	GGA	CCC	TCA	GCC	TGG	GAG	GCC	TGC	CCT	CCC	GCC	CTC	CGT	GGG	CTT	1889
Gly	Gly	Pro	Ser	Ala	Trp	Glu	Ala	Cys	Pro	Pro	Ala	Leu	Arg	Gly	Leu	
			585					590					595			
CAT	CAT	GAC	CTC	CTG	CTC	CGC	AAA	ATG	TCT	TCA	TCG	TCC	CCA	GAC	CTG	1937
His	His	Asp	Leu	Leu	Leu	Arg	Lys	Met	Ser	Ser	Ser	Ser	Pro	Asp	Leu	
		600					605					610				
CTG	TCA	GCA	GCA	CTA	GGG	TCC	CGG	GGC	CGG	GGG	GCC	ACA	GGC	GGA	GCT	1985
Leu	Ser	Ala	Ala	Leu	Gly	Ser	Arg	Gly	Arg	Gly	Ala	Thr	Gly	Gly	Ala	
	615					620					625					
GGG	GAT	CCT	GGC	TCA	CCA	CCT	CCG	GCC	CGG	GGT	GAC	ACC	CCA	CCA	AGT	2033
Gly	Asp	Pro	Gly	Ser	Pro	Pro	Pro	Ala	Arg	Gly	Asp	Thr	Pro	Pro	Ser	
	630				635					640					645	
GAG	GGC	TCA	GCC	CCT	GGC	TCC	ACC	AGC	CCA	GAT	TCA	CCT	GGG	GGA	GCC	2081
Glu	Gly	Ser	Ala	Pro	Gly	Ser	Thr	Ser	Pro	Asp	Ser	Pro	Gly	Gly	Ala	
			650					655						660		
AAA	GGG	GAA	CCA	CCT	CCT	CCA	GTA	GGG	CCT	GGT	GAA	GGT	GTG	GGG	CTT	2129
Lys	Gly	Glu	Pro	Pro	Pro	Pro	Val	Gly	Pro	Gly	Glu	Gly	Val	Gly	Leu	
			665				670						675			
CTG	GGA	ACT	GGA	AGG	GAA	GGG	ACC	TCA	GGC	CGG	GGA	GGA	AGC	CGG	GCT	2177
Leu	Gly	Thr	Gly	Arg	Glu	Gly	Thr	Ser	Gly	Arg	Gly	Gly	Ser	Arg	Ala	
		680					685					690				
GGG	TCC	CAG	CAC	TTG	ACC	CCA	TCT	GCA	CTG	CTG	TAC	AGG	GCT	GCC	GTC	2225
Gly	Ser	Gln	His	Leu	Thr	Pro	Ala	Ala	Leu	Leu	Tyr	Arg	Ala	Ala	Val	
	695					700					705					
ACC	CGA	AGT	CAG	AAA	CGT	GGC	ATC	TCA	TCG	GAA	GAG	GAG	GAA	GGA	GAG	2273
Thr	Arg	Ser	Gln	Lys	Arg	Gly	Ile	Ser	Ser	Glu	Glu	Glu	Glu	Gly	Glu	
	710				715					720				725		
GTA	GAC	AGT	GAA	GTA	GAG	CTG	ACA	TCA	AGC	CAG	AGG	TGG	CCT	CAG	AGC	2321
Val	Asp	Ser	Glu	Val	Glu	Leu	Thr	Ser	Ser	Gln	Arg	Trp	Pro	Gln	Ser	
			730					735						740		
CTG	AAC	ATG	CGC	CAG	TCA	CTA	TCT	ACC	TTC	AGC	TCA	GAG	AAT	CCA	TCA	2369
Leu	Asn	Met	Arg	Gln	Ser	Leu	Ser	Thr	Phe	Ser	Ser	Glu	Asn	Pro	Ser	
			745					750					755			
GAT	GGG	GAG	GAA	GGC	ACA	GCT	AGT	GAA	CCT	TCC	CCC	AGT	GGC	ACA	CCT	2417
Asp	Gly	Glu	Glu	Gly	Thr	Ala	Ser	Glu	Pro	Ser	Pro	Ser	Gly	Thr	Pro	
		760				765						770				
GAA	GTT	GGC	AGC	ACC	AAC	ACT	GAT	GAG	CGG	CCA	GAT	GAG	CGG	TCT	GAT	2465
Glu	Val	Gly	Ser	Thr	Asn	Thr	Asp	Glu	Arg	Pro	Asp	Glu	Arg	Ser	Asp	
	775					780					785					
GAC	ATG	TGC	TCC	CAG	GGC	TCA	GAA	ATC	CCA	CTG	GAC	CCA	CCT	CCT	TCA	2513
Asp	Met	Cys	Ser	Gln	Gly	Ser	Glu	Ile	Pro	Leu	Asp	Pro	Pro	Pro	Ser	
	790				795				800						805	
GAG	GTC	ATC	CCT	GGC	CCT	GAA	CCC	AGC	TCC	CTG	CCC	ATT	CCA	CAC	CAG	2561
Glu	Val	Ile	Pro	Gly	Pro	Glu	Pro	Ser	Ser	Leu	Pro	Ile	Pro	His	Gln	
			810						815					820		
GAA	CTT	CTC	AGA	GAG	CGG	GGC	CCT	CCC	AAT	TCT	GAG	GAC	TCA	GAC	TGT	2609
Glu	Leu	Leu	Arg	Glu	Arg	Gly	Pro	Pro	Asn	Ser	Glu	Asp	Ser	Asp	Cys	
			825					830					835			

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GAC AGC ACT GAA TTG GAC AAC TCC AAC AGC GTT GAT GCC TTG CGC CCC 2657
 Asp Ser Thr Glu Leu Asp Asn Ser Asn Ser Val Asp Ala Leu Arg Pro
 840 845 850

CCA GCT TCC CTC CCT CCA TGAAAGCCAC TCGTATTCCT TGTACATAGA 2705
 Pro Ala Ser Leu Pro Pro
 855

GAAATATTTA TATGGATTAT ATATATATAC ATATATATAT ATATATGCGC CACATAATCA 2765
 ACAGAAAGAT GGGGCTGTCC CAGCCGTAAG TCAGGCTCGA GGGAGACTGA TCCCCTGACC 2825
 AATTCACCTG ATAACTCTA GGGACACTGG CAGCTGTGGA AATGAATGAG GCACAGCCGT 2885
 AGAGCTGTGG CTAAGGGCAA GCCCCTTCCT GCCCCACCCC ATTCCTTATA TTCAGCAAGC 2945
 AACAAAGGCAA TAGAAAAGCC AGGGTTGTCT TTATATTCTT TATCCCCAAA TAATAGGGGG 3005
 TGGGGGGAGG GCGGTGGGA GGGGCAGGAG AGAAAACCAC TTAGACTGCA CTTTTCTGTT 3065
 CCGTTTACTC TGTTTACACA TTTTGCACTT GGGAGGAGGG AGGCTAAGGC TGGGTCCTCC 3125
 CCTCTGAGGT TTCTCAGGTG GCAATGTAAC TCATTTTTTT GTCCCACCAT TTATCTTCTC 3185
 TGCCCCAAGCC CTGTCTTAAG GCCCAGGGGG AGGTTAGGAG ACTGATAGCA TGTGATGGCT 3245
 CAGGCTGAAG AACCGGGGTT CTGTTTAAGT CCCTGCTTTT ATCCTGGTGC CTGATTGGGG 3305
 TGGGGACTGT CCTACTGTAA CCCCTGTGAA AAACCTTGAA AAATAACACT CCATGCAGGA 3365
 AAAAAAAAAA AAAAAAAAAA AAAA 3389

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 859 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Cys Leu His Glu Thr Arg Thr Pro Ser Pro Ser Phe Gly
 1 5 10 15
 Gly Phe Val Ser Thr Leu Ser Glu Ala Ser Met Arg Lys Leu Asp
 20 25 30
 Pro Asp Thr Ser Asp Cys Thr Pro Glu Lys Asp Leu Thr Pro Thr
 35 40 45
 His Val Leu Gln Leu His Glu Gln Asp Ala Gly Gly Pro Gly Gly
 50 55 60
 Ala Ala Gly Ser Pro Glu Ser Arg Ala Ser Arg Val Arg Ala Asp
 65 70 75
 Glu Val Arg Leu Gln Cys Gln Ser Gly Ser Gly Phe Leu Glu Gly
 80 85 90
 Leu Phe Gly Cys Leu Arg Pro Val Trp Thr Met Ile Gly Lys Ala
 95 100 105
 Tyr Ser Thr Glu His Lys Gln Gln Gln Glu Asp Leu Trp Glu Val
 110 115 120

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Pro Phe Glu Glu Ile Leu Asp Leu Gln Trp Val Gly Ser Gly Ala	125	130	135
Gln Gly Ala Val Phe Leu Gly Arg Phe His Gly Glu Glu Val Ala	140	145	150
Val Lys Lys Val Arg Asp Leu Lys Glu Thr Asp Ile Lys His Leu	155	160	165
Arg Lys Leu Lys His Pro Asn Ile Ile Thr Phe Lys Gly Val Cys	170	175	180
Thr Gln Ala Pro Cys Tyr Cys Ile Leu Met Glu Phe Cys Ala Gln	185	190	195
Gly Gln Leu Tyr Glu Val Leu Arg Ala Gly Arg Pro Val Thr Pro	200	205	210
Ser Leu Leu Val Asp Trp Ser Met Gly Ile Ala Gly Gly Met Asn	215	220	225
Tyr Leu His Leu His Lys Ile Ile His Arg Asp Leu Lys Ser Pro	230	235	240
Asn Met Leu Ile Thr Tyr Asp Asp Val Val Lys Ile Ser Asp Phe	245	250	255
Gly Thr Ser Lys Glu Leu Ser Asp Lys Ser Thr Lys Met Ser Phe	260	265	270
Ala Gly Thr Val Ala Trp Met Ala Pro Glu Val Ile Arg Asn Glu	275	280	285
Pro Val Ser Glu Lys Val Asp Ile Trp Ser Phe Gly Val Val Leu	290	295	300
Trp Glu Leu Leu Thr Gly Glu Ile Pro Tyr Lys Asp Val Asp Ser	305	310	315
Ser Ala Ile Ile Trp Gly Val Gly Ser Asn Ser Leu His Leu Pro	320	325	330
Val Pro Ser Ser Cys Pro Asp Gly Phe Lys Ile Leu Leu Arg Gln	335	340	345
Cys Trp Asn Ser Lys Pro Arg Asn Arg Pro Ser Phe Arg Gln Ile	350	355	360
Leu Leu His Leu Asp Ile Ala Ser Ala Asp Val Leu Ser Thr Pro	365	370	375
Gln Glu Thr Tyr Phe Lys Ser Gln Ala Glu Trp Arg Glu Glu Val	380	385	390
Lys Leu His Phe Glu Lys Ile Lys Ser Glu Gly Thr Cys Leu His	395	400	405
Arg Leu Glu Glu Glu Leu Val Met Arg Arg Arg Glu Glu Leu Arg	410	415	420
His Ala Leu Asp Ile Arg Glu His Tyr Glu Arg Lys Leu Glu Arg	425	430	435
Ala Asn Asn Leu Tyr Met Glu Leu Asn Ala Leu Met Leu Gln Leu	440	445	450

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Glu Leu Lys Glu Arg	Glu Leu Leu Arg	Arg Glu Gln Ala Leu Glu	455	460	465
Arg Arg Cys Pro Gly	Leu Leu Lys Pro	His Pro Ser Arg Gly Leu	470	475	480
Leu His Gly Asn Thr	Met Glu Lys Leu	Ile Lys Lys Arg Asn Val	485	490	495
Pro Gln Asn Leu Ser	Pro His Ser Gln Arg	Pro Asp Ile Leu Lys	500	505	510
Ala Glu Ser Leu Leu	Pro Lys Leu Asp	Ala Ala Leu Ser Gly Val	515	520	525
Gly Leu Pro Gly Cys	Pro Lys Ala Pro	Pro Ser Pro Gly Arg Ser	530	535	540
Arg Arg Gly Lys Thr	Arg His Arg Lys	Ala Ser Ala Lys Gly Ser	545	550	555
Cys Gly Asp Leu Pro	Gly Leu Arg Thr	Ala Val Pro Pro His Glu	560	565	570
Pro Gly Gly Pro Gly	Ser Pro Gly Gly	Leu Gly Gly Gly Pro Ser	575	580	585
Ala Trp Glu Ala Cys	Pro Pro Ala Leu Arg	Gly Leu His His Asp	590	595	600
Leu Leu Leu Arg Lys	Met Ser Ser Ser	Ser Pro Asp Leu Leu Ser	605	610	615
Ala Ala Leu Gly Ser	Arg Gly Arg Gly	Ala Thr Gly Gly Ala Gly	620	625	630
Asp Pro Gly Ser Pro	Pro Pro Ala Arg	Gly Asp Thr Pro Pro Ser	635	640	645
Glu Gly Ser Ala Pro	Gly Ser Thr Ser	Pro Asp Ser Pro Gly Gly	650	655	660
Ala Lys Gly Glu Pro	Pro Pro Pro Val	Gly Pro Gly Glu Gly Val	665	670	675
Gly Leu Leu Gly Thr	Gly Arg Glu Gly	Thr Ser Gly Arg Gly Gly	680	685	690
Ser Arg Ala Gly Ser	Gln His Leu Thr	Pro Ala Ala Leu Leu Tyr	695	700	705
Arg Ala Ala Val Thr	Arg Ser Gln Lys	Arg Gly Ile Ser Ser Glu	710	715	720
Glu Glu Glu Gly Glu	Val Asp Ser Glu	Val Glu Leu Thr Ser Ser	725	730	735
Gln Arg Trp Pro Gln	Ser Leu Asn Met	Arg Gln Ser Leu Ser Thr	740	745	750
Phe Ser Ser Glu Asn	Pro Ser Asp Gly	Glu Glu Gly Thr Ala Ser	755	760	765
Glu Pro Ser Pro Ser	Gly Thr Pro Glu	Val Gly Ser Thr Asn Thr	770	775	780

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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCATGAAAGC CACTCGTATT CCT 23

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SBQ ID NO:4:

TCTTTCTGTT GATTATGTGG CGC

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What is claimed is:

1. cDNA coding for mRNA which encodes a leucine-zipper protein kinase.
2. cDNA having a sequence at least about 50% homologous to the nucleic acid sequence set forth in SEQ ID NO: 1.
3. A protein produced from the cDNA of claim 2.
4. A substantially purified, isolated mammalian leucine-zipper protein kinase comprising a protein having a sequence at least about 50% homologous to the amino acid sequence set forth in SEQ ID NO: 2.
5. A pharmaceutical composition comprising leucine-zipper protein kinase in a pharmaceutically acceptable carrier.
6. A polynucleotide probe comprising a nucleic acid sequence substantially homologous to at least a portion of the cDNA of claim 2.
7. A polynucleotide probe comprising a nucleic acid sequence specifically hybridizable to at least a portion of the cDNA of claim 2.
8. A construct comprising a vector and at least a portion of the cDNA of claim 2.
9. The construct of claim 8 further comprising a promoter operably linked to said cDNA.
10. Recombinant host cells transformed with cDNA of claim 2 or a portion thereof whereby said host cells express a leucine-zipper protein kinase.

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11. A method of producing leucine-zipper protein kinase which comprises culturing recombinant host cells wherein said host cells are transformed with cDNA having SEQ ID NO: 2 operably linked to regulatory control sequences which effect the expression of said coding sequence in said transformed host cells and isolating said leucine-zipper protein kinase produced by said host cells.

12. A method of detecting a gene coding for leucine-zipper protein kinase in a sample comprising contacting the sample with a probe of claim 6 or claim 7 under conditions which allow for the formation of a polynucleotide duplex between the probe and said gene, and detecting the presence or absence of a polynucleotide duplex whereby the presence of a polynucleotide duplex indicates the presence of said leucine-zipper protein kinase in said sample.

13. A method of inhibiting the hyperproliferation of neuronal cells comprising contacting said cells with an oligonucleotide substantially complementary to the nucleic acid sequence set forth in SEQ ID NO: 1.

14. A method of inhibiting the formation of protein-protein kinase complexes in a cell comprising contacting said cell with polypeptides having a sequence substantially homologous to at least a portion of the sequence set forth in SEQ ID NO: 2.

15. The method of claim 14 wherein said portion of the sequence set forth in SEQ ID NO: 2 comprises at least amino acids 442 through 468.

16. A method of inhibiting the formation of nucleic acid-protein kinase complexes in a cell comprising contacting said cell with polypeptides having a sequence substantially homologous to at least a portion of the sequence set forth in SEQ ID NO: 2.

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17. The method of claim 16 wherein said portion of the sequence set forth in SEQ ID NO: 2 comprises at least amino acids 442 through 468.

18. A method of inhibiting the formation of protein-protein kinase complexes in a cell comprising contacting said cell with an oligonucleotide substantially complementary to a portion of the nucleic acid sequence set forth in SEQ ID NO: 1.

19. A method of inhibiting the formation of nucleic acid-protein kinase complexes in a cell comprising contacting said cell with an oligonucleotide substantially complementary to a portion of the nucleic acid sequence set forth in SEQ ID NO: 1.

20. A method of treating a mammal suffering from tumors of the central nervous system comprising administering to said mammal a therapeutically effective amount of an oligonucleotide having a sequence substantially complementary to a portion of the sequence set forth in SEQ ID NO: 1.

21. A method of treating a mammal suffering from tumors of the central nervous system comprising administering to said mammal a therapeutically effective amount of a polypeptide having a sequence substantially homologous to at least a portion of the sequence set forth in SEQ ID NO: 2.

22. The method of claim 21 wherein said portion of the sequence set forth in SEQ ID NO: 2 comprises at least amino acids 442 through 468.

[illegible]

SUBSTITUTE SHEET (RULE 26)

	R L E E E L V M R M R R R E E L S H A L D I R E H Y E	430
1388	AAGGAGCTGGAGAGGOCACAACTGTATATGGAAGCTTAATGCOCTCATGTTGCGAGCTGGACTCAAGGAG	
	R K L E R A N N L Y M E (L) N A L M L Q (L) E L K E	454
1461	AGGGAGCTGCTAGGOGAGGCAAGCTTTAGAGGGGCGTGOCAGGCGCTGCTGAGGOCACGCTTTCGCGG	
	R E (L) L R R E Q A (L) E R R C P G L L K P H P S R	478
1534	GCTCTGCTGATGGAAACACATGGGAGAGCTTATCAAGAGAGGAATGTGOCACAGATCTGTCTACCCATAG	
	G L L H G N T M E K L I K K R N V P Q N L S P H S	503
1607	CCAAAGGOCAGATATCTCTCAAGGGGAGCTTTTGCTCTCTAACTAGATGCGAGGCTGAGTGGGGTGGGGCTT	
	Q R P D I L K A E S L L P K L D A A L S G V G L	527
1680	CGTGGGTGTCTAAGGCCCCCCCCCAACAGGAGCGGAGTGCGGGTGGGAGAGGCGGTCAAGGAGGOCAGG	
	P G C P K A P P S P G R S R R G K T R H R K A S	551
1753	CCAGGGGAGCTGTGGGGAGCTGCTGGGCTTGCTACAGCTGTGCTGACGCGGATGAACCTGGGAGGACAGGAG	
	A K G S C G D L P G L R T A V P P H E P G G P G S	576
1826	CCAGGGGGCTTAGGAGGGGGAGCTCTAGGCTGGGAGGCTGCGCTCGGCTCGGCTCGGCTCGGCTCGGCTCGGCT	
	P G G L G G G P S A W E A C P P A L R G L H H D	600
1899	CTCTGCTCGGCAAAATGCTTCTATGCTGCGGAGCTGCTGTGAGAGGAGCTAGGGTTCGGGGGGGGGGGG	
	L L L R K M S S S S P D L L S A A L G S R G R G	624
1972	CCAGGGGGAGCTGGGGAGCTGCGCTCTAGAGCTGCGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	
	A T G G A G D P G S P P P A R G D T P P S E G S A	649
2045	CGCTGGCTCTGAGAGGOCAGTCACTTGGGGGGGAGGAGGGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG	
	P G S T S P D S P G G A K G E P P P P V G P G E	673
2118	GGTGTGGGGCTTCTGGGAGCTGGAGGGAGGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	
	G V G L L G T G R E G T S G R G G S R A G S Q H	697
2191	TGACCGGAGCTGACCTGCTGTACAGGGCTGCGGTCAAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG	
	L T P A A L L Y R A A V T R S Q K R G I S S E E E	722
2264	GGAG	
	E G E V D S E V E L T S S Q R W P Q S L N M R Q	746
2337	TGATATCTCACTCTAGCTGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	
	S L S T F S S E N P S D G E E G T A S E P S P S	770
2410	GCTACCGTGAAGTGGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	
	G T P E V G S T N T D E R P D E R S D D M C S Q G	795
2483	CTCAGAAATCCAGCTGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	
	S E I P L D P P P S E V I P G P E P S S L P I P	819
2556	CACAGGAGCTTCTCAGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	
	H Q E L L R E R G P P N S E D S D C D S T E L D	843
2629	ACTCCAGAGGCTGATGCTTGGGCCCCAGCTTCCCTCTCTCATGAGAGGAGGAGGAGGAGGAGGAGGAGGAGG	
	N S N S V D A L R P P A S L P P *	859
2702	tagagaaatatttatatggatttatatatatacatatatatatatatatgogocacataaatcaacagaaaga	
2775	tggggctgtccagagcgttaagtcaggctcgaggggagactgatccctgacaaattcaactgataaactctagg	
2848	gacactggcagctgtggaatgaatgaggcagagcgttagagctgtggcgaagggcaagccctctctgccc	
2921	accocattctctattcaggcaagcaacaagggcaatagaaagccagggctgtgtcttatattctttatcccc	
2994	aataaatagggggtgg	
3067	cgctttactctgtttacacattttggccttgg	
3140	caggtggcaatgtaactcattttttggccaccatttatcttctctgcccagccctgtcttaaggggccagg	
3213	ggggaggttagggagactgatagcatgtgagctgggctcaggctgaagaaacgggggttctgtttaagtcctgtttt	
3286	atcctgctgctgatttgggggtggggagctgctactgcaacccctgtgaaaaaccttgaaaaataaacctcca	
3359	tgcaggaaaaaataaaaaataaaaaaataaaaaa	

SUBSTITUTE SHEET (RULE 26)

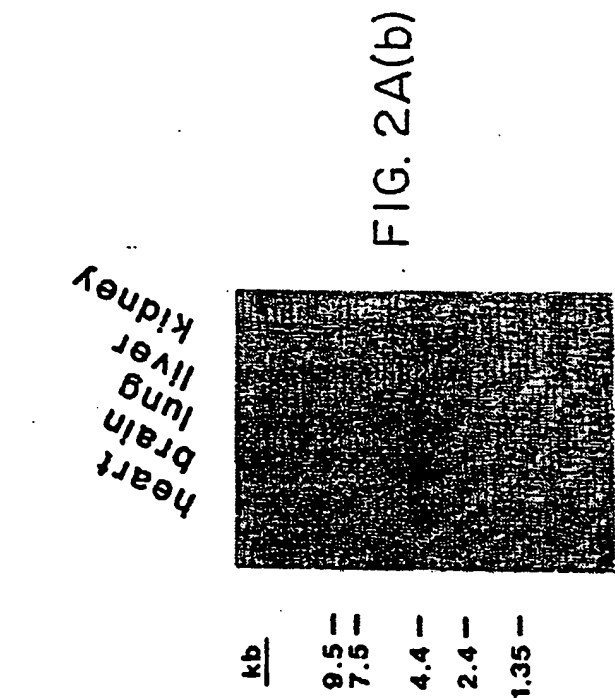


FIG. 2A(b)

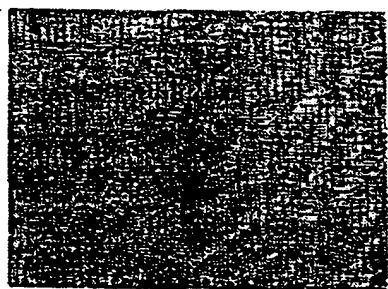


FIG. 2B(a)



2.4 —
1.35 —

FIG. 2B(b)



2.4 —
1.35 —

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/02792

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12N 9/12, 15/54

US CL : 435/194; 536/23.2; 424/93.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/194; 536/23.2; 424/93.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Dialog, APS

search terms: leucine-zipper protein kinase

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
NONE	NONE	NONE

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principles or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

15 MAY 1995

Date of mailing of the international search report

07 JUN 1995

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/02792

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-2, 8-11, 13 and 18-20, drawn to cDNA and first method of use.

Group II, claims 3-5, drawn to a leucine-zipper protein kinase.

Group III, claims 6-7 and 12, drawn to DNA probes and methods of use.

Group IV, claims 14-17 and 21-22, drawn to a method of inhibiting cells using the protein.

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons.

Groups I and II are physically and chemically unrelated compounds, each with separate function and industrial applicability.

Group III is unrelated to the protein of Group II both chemically and physically, also with separate functions. The cDNA of Group I and the probes of Group III are separate, in that they are not necessarily physically the same, and each possess distinct function and industrial applicability from the other. Thus, they lack a corresponding special technical feature.

The method of Group IV does not employ the compounds of Groups I and III, and thus lacks a common special technical feature. The method of Group IV employs the protein of Group II, but the protein has separate function outside this particular method, and the method can be performed with another compound. Since the method does not necessarily require the specific protein, they lack a corresponding special technical feature.

Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.